AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

THIS IS Exhibit 1 referred to in the Statutory Declaration of Francis John Ballard

made before me this 16th Day of February, 2000

B. Dalman, J.P. 17434

B. DALMAN
A Justice of the Peace for the State of South Australia

Abbreviated Curriculum Vitae, F.J. BALLARD, October 1999

1 NAME — Francis John BALLARD

2 BIRTH — 19 June 1940, Penang, Malaysia

3 QUALIFICATIONS

1961 BSc (Hons) (Univ. W.A)
1964 PhD (Univ. W.A)
1984 DSc (Univ. W.A)

4 PRESENT POSITIONS

Managing Director, GroPep Pty Ltd
Director and Chairman, PrimeGRO Pty Ltd
Affiliate Professor in Biotechnology, University of Adelaide

5 PREVIOUS APPOINTMENTS

1965-1966	Research Fellow, Temple University, USA
1966-1969	Assistant Professor of Biochemistry, Temple University, USA
1969-1971	Queen Elizabeth Fellow, CSIRO Nutritional Biochemistry
1971-1972	Senior Research Scientist, CSIRO Nutritional Biochemistry
1972-1977	Principal Research Scientist, CSIRO Nutritional Biochemistry
1977-1980	Senior Principal Research Scientist, CSIRO Human Nutrition
1986-1994	Chief Research Scientist, CSIRO Human Nutrition
1986-1991	Assistant Chief, CSIRO Human Nutrition
1991-1994	CEO, GroPep Pty Ltd
1991-1999	Director, CRC for Tissue Growth and Repair

6 RESEARCH HONOURS and AWARDS

1971	Mead Johnson Award for Research in Nutrition
1975	Edgeworth David Medal
1976	Boehringer Mannheim Medal
1978	David Rivett Medal
1980	LKB Medal
1984	Lemberg Medal
1997	Fellow, Australian Academy of Technological Sciences and Engineering

7 MEMBERSHIPS, COMMITTEES and OTHER ACTIVITIES

1972-1977	Secretary, Australian Biochemical Society
1979-1982	Chairman, Committee on Symposia, International Union of Biochemistry
1979-1985	Editorial Board, Journal of Development Physiology
1979-1994	SIDS Research Committee (SA)
1981-1991	Editorial Board, Proceedings of the Nutrition Society of Australia
1982-1984	President, Australian Biochemical Society
1982-1990	International Committee on Proteolysis
1983-1988	Editorial Board, Journal of Nutrition, Growth and Cancer
1983-1990	Editorial Board, Cell Biology and Toxicology
1983-present	Muscular Dystrophy Association (SA) Research Advisory Committee
1986-1990	Queen Victoria Hospital – Research Advisory Committee
1987-1990	President, Australian Perinatal Society
1988-1990	Bresatec Ltd – Research Advisory Committee
1990-1994	Child Health Research Institute – Research Advisory Committee
1994-present	Editorial Board, Biochemical Journal
1995-present	Editorial Board, International Journal of Biochemistry and Cell Biology
1999-present	Member, Australian Government Biotechnology Consultative Committee

8 PUBLICATIONS Total Number = 290

KEY PAPERS

Ballard, F.J., Hanson, R.W and Kronfeld, D.S (1969) Carbohydrate and lipid metabolism in ruminants. *Fed.Proc. 28*, 218-231.

Philippidis, H., Hanson, R.W., Reshef, L., Hopgood, M.F. and **Ballard, F.J.** (1972) The initial synthesis of proteins during development. Phosphoenolpyruvate carboxykinase mRNA during glucose repression in liver. *Biochem. J.* **126**, 1127-1134.

Tilghman, S., Hanson, R.W., Reshef, L. Hopgood, M.F. and **Ballard, F.J.** (1974) Rapid loss of translatable phosphoenolpyruvate carboxykinase mRNA during glucose repression in liver. *Proc. Nat. Acad. Sci. US* **71**, 1304-1308.

Knowles, S.E and **Ballard, F.J.** (1976) Selective control of the degradation of normal and aberrant proteins in Reuber H35 hepatoma cells. *Biochem.J.* **156**, 609-617.

Gunn, J.M., Clark, M.G., Knowles, S.E., Hopgood, M.F and **Ballard, F.J.** (1977) Reduced rates of proteolysis in transformed cells. *Nature* **266**, 58-60

Ballard, F.J., Knowles, S.E., Wong, S.S.C., Bodner, J.B., Wood, C.M. and Gunn, J.M. (1980) Inhibition of protein breakdown in cultured cells is a consistent response to growth factors. *FEBS Lett.* **114**, 209-212.

Ballard, F.J., Francis, G.L., Ross, M., Bagley, C.J., May, B. and Wallace, J.C. (1987) Natural and synthetic forms of insulin-like growth factor-1 (IGF-1) and the potent derivative, destripeptide IGF-1: biological activities and receptor binding. *Biochem. Biophys. Res. Commun.* **149**, 398-404.

Hopgood, M.F., Knowles, S.E., Bond, J.S. and **Ballard, F.J** (1988) Degradation of native and modified forms of aldolase microinjected into HeLa cells. *Biochem.J.* **256**, 81-88.

Conlon, M.A., Tomas, F.M., Owens, P.C., Wallace, J.C. Howarth, G.S and **Ballard, F.J.** (1995) Long R^3 insulin-like growth factor-I (LR^3IGF-I) infusion stimulates organ growth but reduces IGF-I, IGF-II and IGF binding proteins in the guinea pig. *J. Endocrinol.* **146**, 247-253.

Belford, D.A., Rogers, M.L, Regester, G.O., Francis, G.L, Liepe, I.J., Priebe, I.K. and **Ballard, F.J.** (1995) Milk-derived growth factors as serum supplements for the growth of fibroblast and epithelial cells. *In vitro Cell. Dev. Biol. Anim.* **31**, 750-758.

9 TECHNOLOGY TRANSFER & COMMERCIALISATION

Patents:

Responsible for the preparation and responses to examiners worldwide for 12 patent families including the following ones as an inventor:

"Peptide analogues of mammalian IGF-I": PCT/AU86/00246
"Growth Factor": PCT/AU88/00485
"Method for treating intestinal diseases": PCT/AU91/00031
"Growth-promoting agent": PCT/AU91/00303
"Method of administering IGF-I, IGF-II and analogs thereof to birds": PCT/US93/08279
"Modified milk growth factor": PCT/AU95/00237
"Use of IGF in combination with insulin": PCT/AU95/00422
"Matrix binding factor": PCT/AU99/00292

Commercialisation:

Responsible for initiating, negotiating and finalising all R & D agreements, Licence agreements, and Distribution agreements on behalf of the CRC for Tissue Growth and Repair as well as GroPep Pty Ltd since 1991.

AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

STATUTORY DECLARATION

I, Kari Alitalo, a Research Professor of the Finnish Academy of Sciences, at The Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, P.O.B. 63 (Haartmaninkatu 8) 00014 University of Helsinki, Finland, do solemnly and sincerely declare as follows:

Introduction

1.1 In February 2000 I executed a first statutory declaration (hereinafter referred to as "OKA1" (Opponents, Kari Alitalo, 1st Declaration)) to provide experimental evidence in support of the opposition filed by Ludwig Institute for Cancer Research ("Ludwig Institute") to the issuance of a patent to Human Genome Sciences, Inc., ("HGS") based on HGS's Australian Patent Application No. 696764 ("the opposed application"). That first declaration included a brief summary of my scientific credentials and an introduction in which I set forth some conventional terminology and relevant background information regarding VEGF-C and signal peptides.

1.2 The patent applicant HGS subsequently filed declarations from three scientists, Jennifer Ruth Gamble (hereinafter "AJG1"), Nicholas Kim Hayward ("ANH1"), and Stuart A. Aaronson ("ASA1"). Those declarations take issue with aspects of my first declaration. I note at the outset that HGS filed three additional declarations from three additional scientists (John Stanley Mattick ("AJM1"), Susan Power ("ASP1"), and Tom Rapoport

("ATR1")) that neglected to comment directly on the issues raised and/or experiments described in my first declaration. Therefore, my initial comments in response to HGS's declarations will be directed principally to the criticisms raised in AJG1, ANH1, and ASA1.

1.3 Second, Ludwig Institute also asked me to provide information regarding sequencing analysis of Human Genome Science's VEGF2[1] clone, deposited with the ATCC and referred to in the opposed application (as amended). That sequence analysis will provide helpful information for replying to issues raised by all of the declarants, but especially Dr. Power.

1.4 Third, Ludwig Institute asked me to comment on the relevance of certain experiments that Human Genome Sciences asked Dr. Susan Power to perform, and that were summarized in ASP1.

Reply to criticisms of my initial experiments and first declaration.

A. Initial observations about the weight of evidence.

2.1 I have reviewed all of the comments made in response to my first declaration. The criticisms regarding the experimental design and the data obtained as a result of my expression studies do not cause me to change my opinion as set forth in my first declaration. I note that AJG1, ANH1, and ASA1 fail to offer scientific data in support of their criticisms and fail to demonstrate that expression of VEGF2 according to the opposed application is feasible. Instead, the declarations recite potential shortcomings in the express teachings of the opposed application and potential remedies thereof (AJG1 at 6.5; ANH1 at 3.13-3.20; ASA1 at 16). If an objective scientist were to study all of the experimental evidence initially presented by the opponent and HGS in this opposition, the scientist would conclude that there is one set of experiments (reported in my first declaration) that VEGF2 as taught in the opposed application is not expressed and

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[1] I note that the body of the specification of the opposed application refers to "VEGF2" whilst the claims and the HGS declarants refer to "VEGF-2". I assume that those terms are used in the opposed application and by the HGS declarants to refer to the same thing.

secreted, and no evidence whatsoever to the contrary. In any event, the further experiments conducted in my laboratory that I describe below provide still more evidence in support of the opponent's position.

2.2 Dr. Gamble criticized my first declaration by observing that I already knew, before conducting any experiments, that the 350 amino acid VEGF2 did not have a proper signal sequence. (AJG1 at 7.41.) She is correct that by 1996 we had evidence that the relevant gene encoded a protein of 419 amino acids (not 350) and that the working signal peptide was from the part that was "missing" from the 350 amino acid VEGF2 sequence in the opposed application. If she thinks that this prior knowledge should have been used in my experimental design or that failure to do so represents a fault in the experimental design, then she misunderstands the purpose for my experiments. It is my understanding that the purpose of the opposition proceeding is to evaluate the merits of the HGS patent application, from the vantage point of early 1994, when it was filed. What I or anybody else knew in 1996 from my independent research should not confuse that inquiry, and certainly should not be credited to HGS. In fact the opposed patent application teaches a 350 amino acid VEGF2 and says that this sequence consists of a leader sequence of 24 amino acids and a mature sequence of about 326 amino acids. Since the patent application contained no experimental evidence whatsoever to support that assertion, I ran a set of experiments to see if it was correct or incorrect. The experiments showed that the patent application was wrong. Additional experiments, reported below, confirm that the opposed application was wrong.

B. My initial experiments were patterned from HGS's own teachings.

2.3 As explained in detail by Dr. Rogers in his first declaration, the opposed application taught an incomplete VEGF2 sequence lacking N-terminal amino acid sequences from the VEGF2 cDNA and protein. HGS filed a second application (**Document D44,** hereinafter referred to as "HGS II") in June 1995, which is not the subject of this opposition, but which is relevant because it pertains to a "full length" VEGF2 that is not missing the N-terminal sequences. Example 3 in HGS II provides a method for the expression of recombinant VEGF2 in COS cells. With the exception of the cell line, I generally followed the experimental design taught in Example 3 of HGS II in my first declaration. (For example, HGS II teaches to express VEGF2 with an HA tag fused in-frame to the 3'

end of VEGF2.) I used 293T cells for my expression studies since it was known at the time of the experimentation that the 293T cell line allows greater proteolytic processing and thus enables VEGF-C precursors a greater opportunity to become secreted. This phenomenon was, in fact, pointed out in each of the declarations (AJG1, Paragraph 7.45; ANH1, Paragraph 5.5; ASA1, Paragraph 10). If anything, the criticisms by Drs. Gamble, Hayward, and Aaronson of the experimental design and evidence supplied in my first declaration provides evidence that HGS II fails to teach a viable method for generating the VEGF2 protein. The Patent Office might wish to forward the criticisms provided by HGS's experts to the appropriate examiner handling the HGS II application in Australia.

2.4 Dr. Gamble, Dr. Hayward, and Dr. Aaronson all criticized my initial work for using an HA tag to make the VEGF2 construct. (See, e.g., AJG1 at 7.43 - 7.44; ANH1 at 5.3-5.4 and 5.6-5.10; and ASA1 at 7 - 13.) Use of the HA tag was allegedly taught in the opposed application (see, e.g., page 8, second full paragraph), and, as explained above, was explicitly taught in an Example of HGS II. If the HA tag causes any problems, then it reflects another defect in the teachings of the application and the teachings of HGS II. And, none of the scientists appears to believe that an HA tag causes any problems, according to other parts of their declarations. (See AJG1 at 7.45; ANH1 at 5.5; and ASA1 at 10.) In any event, I repeated the experiments for this declaration and used a VEGF-C antibody to precipitate both VEGF-C and VEGF2, thus answering the criticisms of Drs. Gamble and Hayward. As reported below, the VEGF2 taught in the patent application still was not expressed and secreted.

C. HGS Patent Application 714484 is irrelevant

2.5 In this paragraph I respond to similar allegations of Drs. Gamble, Hayward, and Aaronson, in which all three declarants attempt to obfuscate the problems in the opposed patent application by relying on information in a totally different patent application:

2.5.1 Dr. Gamble expressed the following opinion at AJG1 at 7.45:

> In HGS' Australian Patent No. 714484, a monoclonal anti-HA antibody was used to successfully immunoprecipitate VEGF-2 which had been modified to contain an HA epitope at its carboxy terminus. It is unclear

to me why Dr. Alitalo apparently was not able to isolate VEGF-2 using a His tag at the C-terminus. One explanation may be the type of mammalian cell line used in the experiments . . . .

2.5.2 Dr. Hayward made similar declarations at ANH1 at 5.5:

> HGS scientists have reported the successful isolation of a modified VEGF-2 protein containing an HA tag at its carboxy terminus using a monoclonal anti-HA antibody. (See, HGS Australian Patent No. 714484 and Hu JS et al (1997) FASEB J May;11(6):498-504). These studies were conducted in COS cells, whereas the experiments set forth in Dr. Alitalo's declaration were conducted in 293T cells . . .

2.5.3 Dr. Aaronson made similar declarations at ASA1 at 10:

> The HGS scientists have reported the successful isolation of a modified VEGF-2 protein containing an HA-tag at its carboxy terminus using a monoclonal antibody to HA (See, HGS Australian Patent No. 714484 and Hu J.S. et al. FASEB J. 11 (6): 498-504). However, the HGS studies were conducted in COS cells, whereas Dr. Alitalo's experiments were conducted in 293T cells . . . .

2.6 As Drs. Gamble, Hayward, and Aaronson all know, the HGS patent document 714484 to which they refer is not the opposed application. Instead, document 714484 is the Australian version of the second application (HGS II) that HGS filed after they realized that the VEGF2 in the opposed application was incomplete.[2] Even if the HA tag was used successfully in an experiment in HGS II, that experiment related to 419 amino acid VEGF2, and not to the merits of the opposed application, which taught an incomplete VEGF2. The results reported in my first declaration for VEGF2 were negative because cells cannot express and secrete the incomplete VEGF2 molecule as taught by HGS. Dr.

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[2] The 1997 publication referred to by Dr. Hayward and Dr. Aaronson was published even later than HGS II was filed, and also relates to the 419 amino acid VEGF2.

Gamble and Dr. Hayward's speculation about cell lines is wrong, as shown in the additional experiments that I describe below.

D. **Additional experiments to prove that VEGF2 cannot be expressed and secreted.**

3.1 Ludwig Institute asked me to design and perform further protein expression studies that would address concerns raised by AJG1, ANH1, and ASA1 directed towards my first declaration. The following analyses of VEGF2 expression, proteolytic processing, and secretion profiles provide further support that the opposed application fails to teach a VEGF2 that can be expressed and secreted.

1. **Background/Review**

3.2. VEGF2 taught by HGS in the opposed application corresponds approximately to amino acids 70 to 419 of the human VEGF-C prepro-peptide. Like most complete protein coding sequences, the VEGF2 taught in the opposed application starts with a methionine. However, as analyzed by the SignalP program at Center for Biological Sequence Analysis, The Technical University of Denmark, this protein does not seem to contain a signal sequence (See OKA1 at 7.1). Among the approximately 70 amino acids that are missing from the N-terminus of VEGF2 in the opposed application are the initial approximately 31 amino acid residues that represent the VEGF-C signal sequence, responsible for directing secretion of the polypeptide.

2. **Experimental Procedure**

3.3 Cells and Plasmids:

3.3.1 A principal criticism from the HGS experts was the use of the 293T cell line rather than the COS cell line. Of course, nothing in the opposed application teaches that 293T cells should not be used.[3] However, in order to fairly evaluate the assertions

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[3] Contrary to anything stated or implied by the HGS declarants, the opposed application has no working examples involving COS cells or other cells. The opposed application mentions COS cells among a list of exemplary cell lines at page 15.

made by HGS's experts, I performed parallel expression studies in both cell lines for this declaration. 293T and COS7 cells were grown in DMEM supplemented with 10% fetal bovine serum, glutamine and penicillin/streptomycin.

3.3.2 The polymerase chain reaction (PCR) was employed to construct a DNA fragment that corresponded to amino acids 70 to 419 of prepro-VEGF-C. Amino acid residues 70 to 419 of prepro-VEGF-C corresponds essentially to the full length sequence of the VEGF2 polypeptide described in the opposed application. Nucleotides 559 to 1608 of a VEGF-C cDNA (GenBank accession number X94216) were PCR amplified with the primers 5'-CGCGGATCCATGACTGTACTCTACCCA-3' containing a BamHI site and 5'-CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGGGTACTCGAGGCTCATTTGTGGTCT-3' containing a XhoI site, HA-tag, a stop codon and a XbaI site. The PCR-amplified DNA was cloned into vector pcDNA1(Amp) (Invitrogen) and the resultant vector was designated as VEGF-2(HGS)/pcDNA1.

3.3.3 Also constructed was a VEGF-C/pcDNA1 construct to serve as a positive control for expression and secretion studies. The VEGF-C/pcDNA1 construct contains the full length (419 codon) cDNA sequence of VEGF-C.

3.4 Metabolic labelling:

3.4.1 To address the differences in expression, proteolytic processing, and secretion among cell lines, both 293T and COS cells were selected for the expression study. 293T or COS7 cells were transfected with either VEGF-2(HGS)/pcDNA1 or VEGF-C/pcDNA1. "Mock" transfected cells (transfected with "empty" vector) were used as a negative control.

3.4.2 Forty-eight hours after transfecting the cells with one or the other plasmid, the cells were washed twice with phosphate-buffered saline solution (PBS) and metabolically labeled in MEM medium containing 100 mCi/ml ^{35}S-methionine and ^{35}S-cysteine (Promix, Amersham) over night. The radioactive amino acids (^{35}S-methionine and ^{35}S-cysteine) were introduced into the cell growth medium to assist in the identification of expressed polypeptides in the extracellular medium

and in cell lysates. The cells used would incorporate these radioactive amino acids into nascent polypeptides during protein biosynthesis. The cell growth media after this overnight growth period is referred to as "conditioned media" because it has been conditioned by whatever polypeptides and other molecules the cells have secreted. After the overnight growth period, the conditioned media was harvested and cleared by centrifugation.

3.4.3 In addition to collecting the extracellular media to assay secreted proteins, the cells were lysed in order to assay proteins that were synthesized in the cells but not secreted. After washing for three times with ice cold PBS, the cells were lysed in ice cold RIPA-buffer (150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris) supplemented with 0.01 U/ml aprotinin, 1 mg/ml leupeptin and 1 mM PMSF, and the lysate was cleared by centrifugation. These latter ingredients were protease inhibitors, to prevent proteolytic degradation of proteins following the lysis step.

3.5 Immunoprecipitation:

Immunoprecipitation experiments were conducted to identify the presence of the various VEGF-C or VEGF2 polypeptides in the conditioned media or cell lystates.

3.5.1 For immunoprecipitation, the conditioned media from cell cultures were supplemented with BSA and Tween 20 to final concentrations of 0.5% and 0.02%, respectively. The different VEGF-C or VEGF2 peptides were immunoprecipitated with polyclonal antibodies raised against a synthetic peptide corresponding to amino acid residues 104-120 of the VEGF-C prepro-peptide (Antisera 882, reported in Document D71, Joukov *et al.*, 1997) at 4°C overnight. As an additional check for the presence of VEGF2 peptides, the conditioned medium and the lysates of the VEGF-2(HGS)/pcDNA1 or mock transfected COS7 cells were also immunoprecipitated with 1 mg/ml monoclonal anti-HA-antibodies (H.A.11, BabCO).

3.5.2 The immunocomplexes were then precipitated with protein A-Sepharose and washed 2-3 times with 1 X binding buffer (0.5% BSA, 0.02% Tween20 in PBS)

and once with PBS at 4°C. The proteins were analyzed by SDS-PAGE in a 12.5% gel under reducing conditions.

3. Experimental Results

3.6 The immunoprecipitated proteins were analyzed by SDS-PAGE on a 12.5% gel under reducing conditions. An autoradiogram of the SDS-PAGE analyses is attached hereto as Exhibit 1.

3.6.1 293T cells (A) or COS7 cells (B and C) were transfected with expression vectors coding for VEGF2 (ie., VEGF-2(HGS)/pcDNA1) or VEGF-C (ie., VEGF-C/pcDNA1). When 293T or COS7 cells are transfected with the VEGF-2(HGS)/pcDNA1 construct, no VEGF2 protein can be detected in the conditioned medium (Exhibit 1, panel A and B, lane 1). These lanes look very much like the "mock" transfected controls in lane 3. Several polypeptides expressed from the VEGF-C/pcDNA1 construct were identified (Exhibit 1, panel A and B, lane 2). The dark band of approximately 30kDa corresponds to a processed form of VEGF-C in which the C-terminal propeptide has been cleaved off. The approximately 21kDa band represents the fully processed form of VEGF-C from which both N- and C-terminal propeptides have been removed. As previously reported, the processing of VEGF-C was less efficient in COS7 cells (Exhibit 1, panel B, lane 2) than in 293T cells (Exhibit 1, panel A, lane 2) (See **Document D71**, Joukov *et al., EMBO J., 16*: 38998-3911(1997)).

3.6.2 The conditioned media and the cell lysates of the COS7 cells transfected with VEGF-2(HGS)/pcDNA1 construct or empty vector ("mock") were also subjected to immunoprecipitation with monoclonal anti-HA antibodies, but no VEGF2 polypeptides could be detected when the immunoprecipitates were analysed by SDS-PAGE (Exhibit 1, panel C).

4. Conclusions

3.7 VEGF-2 as taught in the opposed application cannot be produced as an expressed and secreted protein. This is evident from the inability of VEGF2 as taught in the opposed application to be immunoprecipitated from conditioned media of either COS cells or 293T

cells. Taking into consideration what is now known about the gene corresponding to VEGF2, it is clear that one reason for this defect is that VEGF2 taught in the opposed application lacks a signal peptide, so it is not secreted. The experiments reported herein also rebut the inference by Dr Rapoport (ATR1 at 15) that residues 70-419 of VEGF2 provide sufficient information for expression, proper processing and secretion.

3.8 We now know that when cells express the full length prepro-VEGF-C, they secrete the resultant protein, which is proteolytically processed. This observation was confirmed by these experiments: as expected, VEGF-C protein products are readily detected in conditioned media from both 293T and COS7 cell lines that were transfected with the full length VEGF-C construct.

3.9 Even though VEGF2 as taught in the opposed application lacks a signal peptide to direct its secretion, we now know that VEGF2 is not really an intracellular protein, either. Since VEGF2 is not a normal intracellular protein, it is likely rapidly degraded in cells, if the truncated protein is synthesized at all. Ineffective production and rapid degradation are two possible explanations why no VEGF2 peptides were detectable in cell lysates of cultured cells transfected with VEGF-2(HGS)/pcDNA1.

3.10 The results of these experiments completely confirm and validate the experiments described in my first declaration, namely, that the 350 amino acid VEGF2 taught by HGS in the opposed application cannot be expressed and secreted as described in the opposed application because it lacks a true signal peptide.

3.11 The results of these experiments eliminate any criticism that the cell lines used for expression influenced the results. VEGF2 as taught in the opposed application cannot be expressed and secreted in either COS cells or 293T cells, whereas full length prepro-VEGF-C can be expressed and secreted in either cell type.

3.12 The results of these experiments eliminate any criticism that the antibody used for identification of polypeptides affected results, because an identical antisera was used for VEGF2 and VEGF-C. The polyclonal antisera raised against amino acids 104-120 of prepro-VEGF-C would have recognized either polypeptide (if it were present) because the recognition sequence for the antisera is present in both the VEGF-C and the VEGF2

sequence. (The results with the anti-HA tag antibody serve only to confirm that VEGF2 as taught in the opposed application is defective for expression and secretion.)

Sequencing the VEGF2 clone that HGS deposited with the ATCC

4.1 The opposed application was originally filed with a blank reference to a deposit with the American Type Culture Collection, which HGS eventually amended to specify ATCC Accession Number 75698, deposited 4 March 1994. (See page 5.) The application also states that the sequence of the polynucleotides contained in the deposited materials are controlling in the event of any conflict with the description of the sequence in the application. (See page 9.) Many of HGS's declarants have made representations concerning what the HGS application would allegedly have taught them, and the nature of the deposited clone is important for assessing the validity of their declarations, as I discuss below and as Drs. Rogers and Ballard also discuss.

4.2 My laboratory obtained a sample of ATCC clone 75698 directly from the ATCC. I attach hereto as Exhibit 2 a copy of original paperwork from the ATCC that accompanied the clone that was shipped to me. My laboratory sequenced the clone using standard laboratory techniques.

4.3 The VEGF2 sequence of ATCC clone 75698 begins as follows . . . .

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          10                  30                  50
ggcacgagcAGAGAACAGGCCAACCTCAACTCAAGGACAGAAGAGACTATAAAATTTGCT
        R  E  Q  A  N  L  N  S  R  T  E  E  T  I  K  F  A
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I have distinguished the parts of the clone that do not correspond with VEGF2 sequence using lower case letters. (This sequence presumably corresponds to sequence from the cloning vector in which the VEGF2 cDNA was inserted when deposited with the ATCC). A comparison between the sequence of the deposit and the VEGF2 sequence in Figure 1 of the opposed patent application shows that the deposited VEGF2 begins with nucleotide 142 or 143 in Figure 1. The first VEGF2 amino acids encoded by the deposited VEGF2 clone are REQANL . . . ., i.e., the clone begins with position 25 of the approximately 350 amino acid VEGF2 in the patent application. In other words, the HGS scientists deposited a cDNA that contained only *the mature* (as taught in the opposed application) VEGF2 of about 326 codons, as taught in the opposed application. The HGS scientists failed to deposit a VEGF2 containing the first approximately 24 codons, which they taught were the leader sequence of VEGF2. (And, compared to the true 419 amino acid VEGF2 taught in the HGS II application, above 93 amino acids are missing from the deposit.)

Reply to Susan Power's Declaration

5.1 Perhaps in response to my first declaration in which I demonstrated that VEGF2 cannot be expressed and secreted as taught in the opposed application, HGS filed a declaration of Susan Power (ASP1) in which she describes some expression experiments of her own. Other HGS declarants speak approvingly of Dr. Power's experiments. (See, e.g., ASA1 at 15 - 22.) This section of my declaration provides an analysis of Dr. Power's experimental work as it relates to the opposed application.

5.2 Dr. Power describes her instructions from HGS as follows: "The Patent Attorneys for Human Genome Sciences (HGS) requested that I perform the following experiments in order to determine whether the 350 amino acid form of VEGF-2 (corresponding to residues 70 to 419 of the 419 amino acid form of VEGF-2) fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells." ASP1 at 2. She was not instructed to repeat any particular teachings in the opposed application, or to use materials or methods described in the application.

5.3 The experiments that HGS asked Dr. Powers to perform may be an interesting scientific curiosity, but they have nothing to do with the teachings in the opposed patent

application. The opposed application teaches the reader that the VEGF2 of about 350 amino acids *already* consists of a leader sequence (i.e., a signal sequence) representing the first approximately 24 amino acids, and a mature protein of 326 amino acids. (See, e.g., page 5 of opposed application; see also OKA1 at 2.1 - 3.3.) However, HGS apparently did not ask her to run such an experiment, or to report the results of such an experiment if she ran it.[4] Scientists in 1994 or today would not have had any reason to express a protein that already contained a signal sequence using a method that involved attaching a second, heterologous signal sequence (e.g., Dr. Power's Ig Kappa signal sequence) to the beginning of the natural signal sequence. Therefore, it is not clear to me what basis there is in the patent application for instructing Dr. Power to attach a heterologous signal sequence to 350 amino acid VEGF2. Dr. Power's experiments are not a replication of any example in the opposed application or a reasonable extension of any of its teachings. Dr. Power was not asked to practice the teachings in the application, but rather, to use her present knowledge to design experiments unrelated to the patent application, using materials and methods that were available in 1994.

5.4 Dr. Powers states that, for starting materials, she used nucleotide sequences obtained directly from the ATCC and says that ATCC Deposit No. 75698 contains the nucleotide sequence encoding the 350 amino acid form of VEGF2. See ASP1 at 5. I find this statement very confusing because, as I indicated above, this ATCC clone does not encode 350 amino acids. Perhaps HGS supplied Dr. Power with the 350 amino acid form of VEGF2, and mistakenly led her to believe that the clone was the same as the deposit. The fact that the ATCC clone does not even contain the first twenty-four amino acids further confirms that HGS considered those amino acids to be the signal peptide, and thought that those amino acids should be removed. The opposed application did not teach to attach a foreign signal sequence to the 350 amino acid sequence.

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[4] As I report herein and in OKA1, I have run that experiment and shown that the teachings in the opposed application are wrong.

5.5 Dr. Power describes the antibody she uses as one "which recognizes the precursor form and the processed form of VEGF2." ASP1 at 3 and 13. This statement is confusing because it is unclear what "precursor" and "processed" refer to. For example, we know from experiments in our laboratory that the 419 amino acid prepro-VEGF-C (the precursor) is processed by removal of a signal peptide, removal of a large C-terminal BR3P domain representing almost half of the protein, and, to produce a fully processed VEGF-C, removal of still another N-terminal pro-peptide. **See Document D71.** Neither of HGS's patent applications relating to VEGF2 teach such processing. The opposed application teaches that VEGF2 is a 350 amino acid precursor with a 326 amino acid mature protein. As we now know that is not correct. Dr. Power further confuses this issue by adding an Ig Kappa signal peptide to the 350 amino acid VEGF2.

5.6 Dr. Power summarizes the results of her experiments in paragraph 15. As I explain above, this should be ignored, because the experiments that she ran are unrelated to the teachings in the patent application. One additional observation regarding her results is that she makes specific mention of a doublet of approximately 30 kDa being present in the medium from the cells. It is worth noting that the opposed application makes no mention of this species of polypeptide, or of a method of making it, or that one should expect to achieve it.

Summary

6.1 The experiments that I report herein confirm my first set of experiments (reported in OKA1) and establish that VEGF2 as taught in the opposed application cannot be expressed and secreted. None of the declarations filed by HGS provide any experimental evidence to refute this fact. To the extent that the HGS declarations offered any criticisms of my first set of experiments (legitimate or otherwise), I have addressed them in my second set of experiments. To the extent that the HGS declarations (e.g., Dr. Power) offer any experimental evidence, such evidence is irrelevant because it is not based on teachings in the opposed patent application.

AND I MAKE this solemn declaration by virtue of the Statutory Declarations Act 1959, and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DECLARED at Helsinki

this 24th day of September 2001

Kari Alitalo

BEFORE ME:

(Signature of Witness)

JUKKA HEIKKILÄ
Notary Public

AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

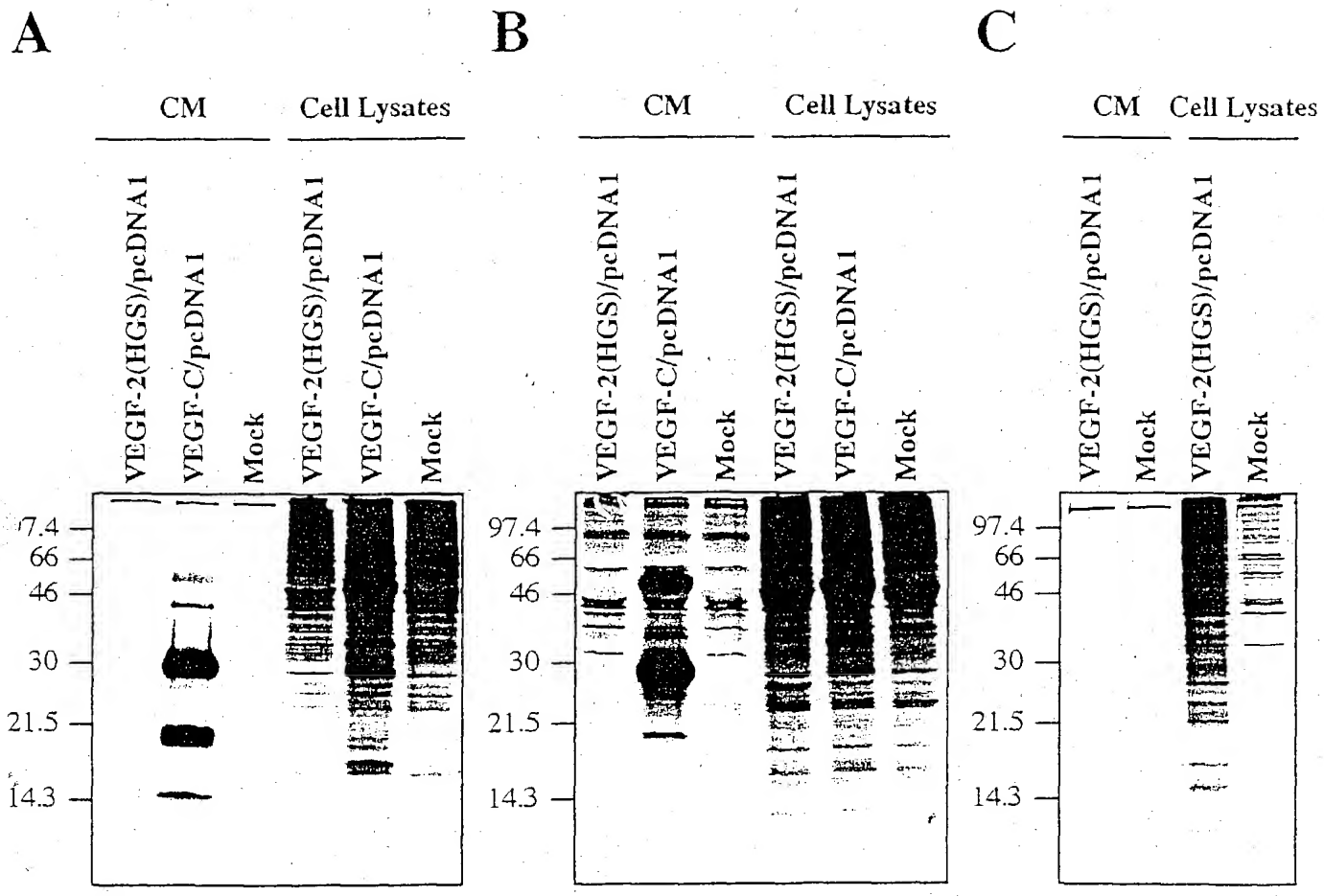
THIS IS Exhibit 1 referred to in the Statutory Declaration of Kari Alitalo

made before me

DATED this 24th Day of September, 2001

.....

Witness JUKKA HEIKKILÄ
Notary Public


A
B
C
CM
Cell Lysates
VEGF-2(HGS)/pcDNA1
VEGF-C/pcDNA1
Mock
97.4
66
46
30
21.5
14.3

AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

THIS IS Exhibit 2 referred to in the Statutory Declaration of Kari Alitalo

made before me

DATED this 27th Day of September, 2001

HELSINGIN MAISTRAATTI MAGISTRATEN I HELSINGFORS

.....

Witness JUKKA HEIKKILÄ
Notary Public

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5. Helsingi ssa
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6. 24 päivänä syys kuuta 2001
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7. Hans Sevelius, julkinen notaari
   av

8. No 8965
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